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Increased breath ethane and pentane concentrations in currently unmedicated patients with schizophrenia

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ABSTRACT

Schizophrenia is a common and debilitating mental illness. The disorder is thought to be developmental in origin, with oxidative stress being implicated as possible pathophysiological mechanism. Breath alkanes provide a non-invasive means to assess oxidative stress, with ethane levels reportedly increased in medicated patients with schizophrenia. It is possible, however, that the psychotropic medications used to treat the disorder result in elevated breath ethane levels. We have therefore measured the concentration of ethane and pentane, markers of oxidative stress, in the breath of currently unmedicated patients with schizophrenia. Alveolar breath samples were collected, applied to thermal desorption tubes, and analyzed using a combination of two-stage thermal desorption, gas chromatography and mass spectrometry. Compared to healthy controls ethane and pentane levels were found to be elevated in patients with schizophrenia, while levels of butane were normal. Our data support the notion that oxidative stress is increased in schizophrenia and that this is unlikely to be a consequence of antipsychotic medications. In addition, breath alkane analysis may represent a rapid and non-invasive means to monitor oxidative stress occurring in schizophrenia.

1. INTRODUCTION

Schizophrenia is a serious mental illness which first presents in late adolescence or early adulthood. [1] The disorder, which affects approximately 1% of the population, is characterized by a range of symptoms including delusions, hallucinations, disordered thoughts and avolition. [1,2] Current treatments have limited efficacy in many patients, with the development of more effective therapies being hampered by a lack of understanding of the pathophysiological mechanism (s) at work. [1] An emerging hypothesis states that disordered oxidative metabolism leads to the brain dysfunction which underlies the disorder. [3] This process, termed oxidative stress, comprises the reactions between metabolically generated free radicals (highly reactive molecules possessing unpaired electrons) and cellular constituents such as DNA, lipids and proteins. [4] The reactions are generally deleterious, leading to a loss of, or altered, function of the cell which, if severe enough, can result in cell death. [4] To combat this process cells possess various antioxidant defenses the purpose of which is to detoxify free radicals. These defenses include enzymes, such as superoxide dismutase, glutathione peroxidase and catalase, and antioxidant compounds, such as vitamin E, which preferentially react with free radicals hence preserving other cellular constituents. [4] As such the degree of oxidative stress a cell experiences is determined by the balance between oxidative and anti-oxidative factors.

In schizophrenia, a number of lines of evidence have suggested that oxidative stress is abnormal [3,5,6]. Since direct measurement of the short lived and reactive free radicals is extremely difficult, most information regarding oxidative stress in schizophrenia derives from the assay of specific components of the anti-oxidant system and/or measurement of the chemical products of oxidative damage. While generally supportive, when examined in detail the findings are somewhat inconsistent. For example, peripheral superoxide dismutase activity, an enzyme which detoxifies superoxide radicals, is reported to be either decreased, increased or unchanged, with similar variability of results being reported for catalase and glutathione peroxidise. [3,5-13] It is notable, however, that when the system is considered as a whole, using methods which assess total antioxidant capacity, a consistent reduction in antioxidant activity in schizophrenia

emerges, indicating an overall elevated susceptibility to free radical damage in the disorder. [14-16] This is supported by several reports of increased levels of DNA, protein and lipid oxidation products in patients with schizophrenia. [17-21] Moreover, the use of an animal model of oxidative stress has indicated that free radical over-activity effects brain development and function, supporting the biological plausibility of oxidative stress playing a role in the disease. [22,23]

As such, monitoring oxidative stress in patients may have some clinical utility. Relating the normally utilised peripheral measures of oxidative stress to that in the brain is challenging however, although evidence of oxidative damage has been detected post mortem in the cortex and hippocampus of patients with schizophrenia. [17,18,21] A recent study has correlated an index of systemic oxidative stress with changes in brain metabolism investigated using *in vivo* ^{31}P magnetic resonance spectroscopy. [24] The marker utilised was ethane, a terminal product of the oxidation of omega-3 polyunsaturated fatty acids [4], which has been shown to be elevated in schizophrenia in two previous studies [25,26], a finding supportive of the occurrence of increased oxidative stress. The other class of polyunsaturated fatty acids, omega-6 fatty acids, also give rise to an alkane oxidation product which differs from that of omega-3 fatty acids, this being pentane [4]. A generalised increase in oxidative stress in schizophrenia would predict that breath concentrations of pentane should be increased although the status of this compound in schizophrenia is currently unknown. Since ethane and pentane are both highly volatile they have the useful feature of equilibrating rapidly into the bloodstream, and subsequently crossing the alveolar membrane to be exhaled in the breath where they can be detected using a variety of analytical approaches including gas chromatography and various forms of chemical ionisation mass spectrometry. [27-29] So-called breath analysis is an emerging methodology which, being non-invasive and rapid, is ideally suited to clinical monitoring. [30] Given the correlation of breath ethane with brain metabolism [24], measuring the breath concentration of this compound may represent a useful means to examine oxidative stress in schizophrenia. Studies utilising breath ethane in schizophrenia should be considered preliminary, however, since only medicated patients with the disorder have been included. Indeed, recent reports have highlighted that pharmaceuticals can alter the chemical makeup of breath, and in fact can give rise to volatile metabolites raising the possibility that ethane may derive directly from such medications. [31,32] In addition, some antipsychotic medications, in particular clozapine and haloperidol, may actually cause increased oxidative stress [33-36], an effect which could again result in altered breath ethane abundance. As such, it presently cannot be ruled out that elevated breath ethane in schizophrenia is due to medication. In this paper we have therefore investigated breath concentrations of both ethane and pentane, as well as butane as a comparator, in a currently un-medicated population of patients with schizophrenia.

2. METHODS

2.1. Participants

Subjects were recruited by invitation having given written informed consent under a protocol approved by the institutional ethics committee. Patients with schizophrenia all had a clinical diagnosis of schizophrenia (n = 28) according to DSM-IV criteria. [37] Patients had not been in receipt of psychotropic medication for three weeks prior to participating in the study but had received medication prior to that (no patients were withdrawn from their medication for the purpose of this study; rather non-compliance with treatment had occurred). Healthy controls (n = 15) had neither history of mental illness nor any medicinal intake of any kind for the previous two weeks, and had no history of any psychotropic drug intake. Almost all subjects were smokers with only four non-smokers in the schizophrenia group and two in the healthy control group with the rate of smoking not differing between groups (χ^2 test; $P > 0.05$). No subject had smoked within the 2 hours prior to breath collection. No subjects had any potentially confounding medical illness. The age of patients with schizophrenia (33 ± 6 years [mean $\pm$ sd]) did not differ significantly (two-tailed, unpaired t-test; $P > 0.05$) from that of the healthy control group (34 ± 7 years). In addition the proportion of males to females in each group (15 females/13 males in the schizophrenia group vs. 8 males/7 females in the healthy control group) did not differ significantly (χ^2 test; $P > 0.05$).

2.2. Breath Sampling

Seated subjects were asked to breathe normally for 3 breaths and then exhale fully into a collection bag constructed in our laboratory. The collection bag was made from PTFE with dimensions of approximately 10 cm × 15 cm and a volume of approximately 200 ml. The bag had both an inlet and outlet, with air from the inlet entering the bag via a unidirectional flow valve, while the outlet tube had a constricting clip attached with which the bag could be sealed. As the subject exhaled the bag inflated and breath passed through the bag to the outlet. When the subject had exhaled fully the bag therefore contained the final 200 ml of exhaled 'alveolar' breath. A similar collection method was also used for ambient air samples except that a syringe was used to inflate the bag.

2.3. Alkane Analysis

Alkane analysis was performed as previously described with minor modifications. [24-26,29] The collection bag was connected via the outlet to a 130 ml PTFE syringe and a volume of gas removed. The syringe was then used to apply the gas sample to a thermodesorption tube (Markes Instruments, UK) containing Carbotrap 300 (Markes Instruments, UK), prior to analysis using Perkin-Elmer autosystem XL equipped with a Turbomass mass spectrometer (Perkin-Elmer, UK). The absorbed gases were desorbed at 320°C onto a cold trap held at 5°C. Secondary desorption at 350°C released the volatiles into a 2 ml min^{-1} stream of helium onto a 30 m × 0.32 mm PLOT GQ column. The initial GC oven temperature of 45°C was maintained for 10 min and increased at 200°C at a rate of 14°C min^{-1}. Eluted gases were detected by electron ionisation mass spectrometry with ethane eluting at 2.6 min, butane at 9.6 min and pentane at 12.1 min, and quantified by comparison with a standard curve constructed using a C1-C6 alkane mix (Supelco, UK).

3. RESULTS

Breath samples were collected from 28 patients with schizophrenia who have received no psychoactive medications for at 2 - 3 weeks prior to sampling, and from 15 healthy controls, and assayed for ethane, butane and pentane. As is observed for other trace gases in breath [38,39], and for breath alkanes in our previous study [26], ethane, butane and pentane concentrations followed an apparent log normal distribution (Lilliefors test for normality of log transformed data; $P > 0.05$ for all groups). Due to this finding the log of the concentration was used in the statistical analysis of the data. Ethane and pentane concentrations were elevated in the breath of patients with schizophrenia compared to healthy controls (two-tailed, unpaired t-test; $P < 0.01$). Butane concentrations did not differ significantly between the two groups (two-tailed, unpaired t-test; $P > 0.05$). Gender had no statistically significant effect upon the concentrations of either gas (two-tailed, unpaired t-test; $P > 0.05$). Age did not correlate significantly ($P > 0.05$) with concentrations of each alkane (data not shown). Butane concentrations did not correlate significantly ($P < 0.05$) with ethane or pentane concentrations in either the schizophrenia (**Figure 2**) or healthy control groups (Pearson correlation coefficient = 0.06), but ethane concentrations were correlated with pentane concentrations in the schizophrenia group (Pearson correlation coefficient = 0.59; $P < 0.001$) but not in the healthy control group (Pearson correlation coefficient = 0.10; $P > 0.05$). Ambient alkane concentrations were below 1 PPBV for each gas, close to the limits of detection of the assay

(approx. 0.1 PPBV), and did not differ significantly between the schizophrenia and healthy control groups (t-test; $P < 0.05$).

4. DISCUSSION

Our findings confirm previous reports [25,26] of elevated breath ethane concentrations in patients with schizophrenia in a different population indicating elevated oxidative damage to omega-3 PUFA. The breath concentrations observed are similar to that found in our previous investigation of schizophrenia [25,26,29], and for healthy controls are similar to that reported by other investigators. [40] In addition, we have also observed increased breath pentane concentrations in the breath of patients with schizophrenia, suggesting that omega-6 PUFA are similarly affected. A common mechanism underlying increased breath ethane and pentane, that of oxidative damage of PUFA, is supported by the correlation between the abundance of each alkane in the schizophrenia group (no significant correlation was observed in the healthy control group but this may be due to the rather low breath alkane concentrations <1 PPB being closer to the limits of detection for the assay). Indeed, reduced levels of PUFA have been reported to occur in schizophrenia (for example see reference 41), an observation which may be due to elevated oxidative damage of these fatty acids in the disorder. On the other hand, ethane concentrations are not reported to be correlated with erythrocyte omega-3 PUFA abundance in
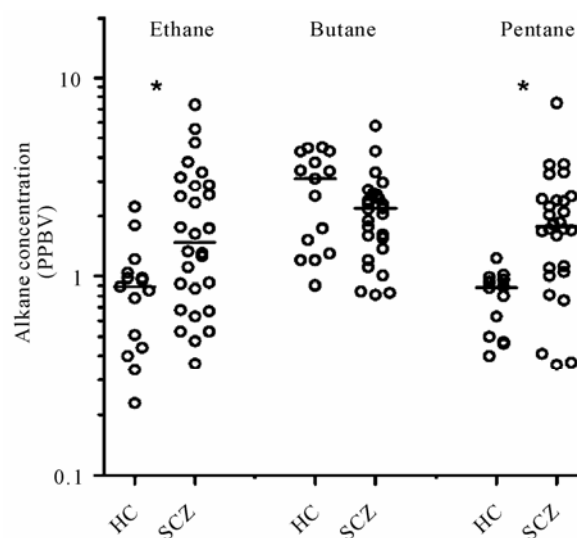
Figure 1. Breath concentrations of ethane, pentane and butane were measured in patients with schizophrenia (SCZ) and healthy controls (HC). Note the logarithmic scale of the Y-axis. The bar indicates the median value. SCZ and HC groups were compared using an unpaired t-test with *indicating P < 0.01. PPBV – parts per billion by volume.
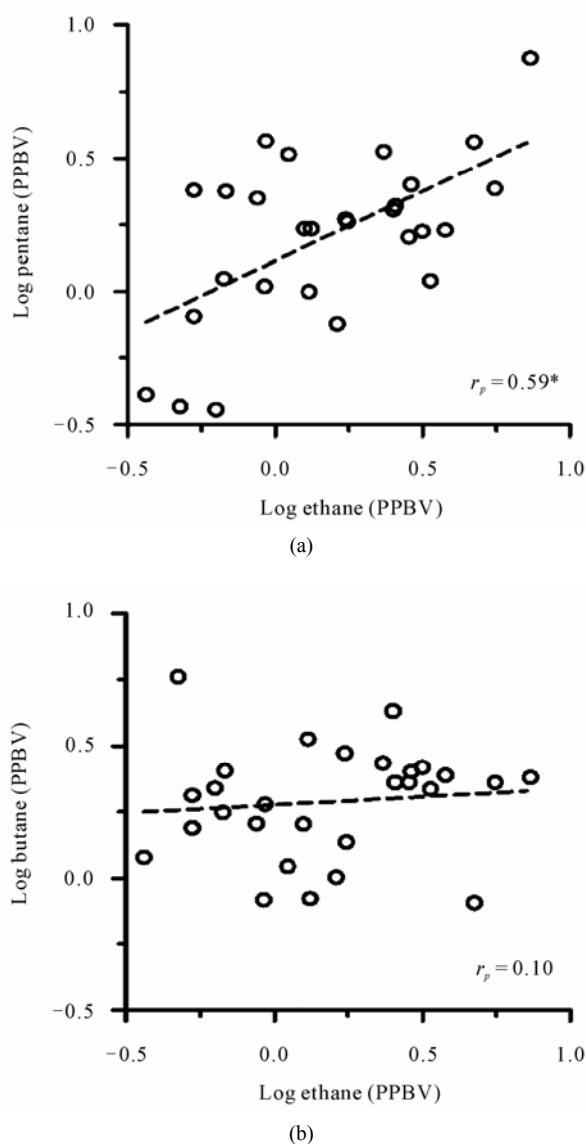
Figure 2. Correlation between alkane concentrations in breath in patients with schizophrenia. The log breath concentrations of (a) pentane or (b) butane are shown plotted against the log concentration of ethane in parts per billion by volume (PPBV). The dashed line shows the 'best fit' linear regression line along with the Pearson correlation coefficient with *indicating $P < 0.001$.

medicated patients [26]. This suggests that other factors, such as dietary intake, may also underlie reduced fatty acid levels in addition to elevated oxidative stress [42] and that there is not a simple relationship between breath alkane concentrations and the abundance of their precursor lipids.

The magnitude of the increase in breath alkane concentrations is similar to that observed in medicated patients [26], even though our participants were unmedicated for at least 3 weeks prior to breath sampling. As such, increased breath ethane and pentane concentrations are likely not derived directly from any administered drugs, although we cannot rule out a longer term effect of any drug upon cellular metabolism given that the participants were not drug-naïve. Such findings are in agreement with data obtained using other experimental approaches which also suggest that psychoactive medication is not an important mediator of oxidative stress in patients. [8,43]

It is important to note that breath butane concentrations were normal in schizophrenia, and found to be uncorrelated with ethane or pentane concentrations. We had previously observed increased breath butane concentrations in schizophrenia, but reasoned that this finding was due to elevated ambient butane abundance in the sampling room used for patients, leading us to conclude that breath butane has an exogenous source. [26] However, ambient butane concentrations were very low in the current study (< 1 PPBV) and breath butane concentrations were routinely above ambient. This does suggest a possible endogenous source for butane, as revealed by low ambient gas concentrations, although the mechanism leading to its production is presently unknown. Interestingly, *in vitro* chemical oxidation of erythrocytes does give rise to butane production although the chemical source is unknown [44-46]. Nevertheless, the lack of correlation with butane, as well as reports that ambient $C1 - C4$ alkane concentrations are correlated in polluted environmental air [47], is supportive of breath ethane and pentane are predominantly endogenous metabolic products (at least when sampled in the absence of significant alkane air pollution as in the current investigation). Notably, breath alkane concentrations were found to follow a log normal distribution, similar to that observed for other breath gases. [38,39]

Most of our subjects were smokers, the rate of which did not differ between the schizophrenia and control groups in this Indian sample. Such a finding differs from that in Western nations but is consistent with a previous report of cigarette use in this country [48]. The lack of a differential smoking rate between the two groups is an important consideration since there reports that smoking increases breath ethane levels although this is not a consistent finding [49,50]. We did not, however, assess the number of cigarettes smoked per day and hence cannot rule out that the schizophrenia group had a higher exposure to tobacco product use than the control group. We have previously reported, however, that there is no correlation between tobacco exposure and ethane concentrations in patients with schizophrenia [26], which supports our conclusion that increased breath alkane concentrations in schizophrenia are consequent to a metabolic change associated with the illness.

Our data therefore offer further evidence for the occurrence of elevated oxidative stress in schizophrenia. Our findings also support the use of breath ethane and/or pentane concentration measurements as a means to monitor oxidative stress in schizophrenia. An examination of **Figure 1**, however, indicates that elevated ethane or pentane concentrations do not occur in all patients with schizophrenia, with many having breath concentrations close to the lower end of the control range. As such elevated ethane and pentane concentrations, and possibly oxidative stress in general, may not be a universal feature of the illness. It remains a possibility, however, that breath ethane and pentane are related to symptom severity in drug-free patients, as are other oxidative markers [43], although this was not addressed in the present study. It should be noted, however, that breath ethane concentration was not correlated with positive or negative symptoms severity in chronic, medicated patients with schizophrenia. [26] In addition to symptom severity this study also did not consider the role of diet which may differ between healthy controls and patients with schizophrenia, thereby representing a possibly confounding factor. For example, McCreadie and colleagues have reported that dietary intake of antioxidants is lower in patients with schizophrenia, a difference which could explain higher breath alkanes in the disorder. [51] Future studies could address this matter by incorporating dietary questionnaires and/or the measurement of systemic antioxidant levels.

It is presently unclear whether increased ethane and pentane concentrations and/or oxidative stress are of primary aetiological importance or are an epiphenomena. Nevertheless, a presumed increase in oxidative stress is not desirable, having been implicated in a variety of common disorders such as atherosclerosis, cancer and diabetes. [52-54] Interestingly, patients with schizophrenia have a higher risk of death from some forms of cancer, heart disease and diabetes. [55-57] One may therefore speculate that is related to increased oxidative stress in the disorder. For this reason it may be desirable to reduce oxidative stress as a precautionary measure by, for example, improving dietary antioxidant consumption, as a means to improve general health.

In summary, we have found that concentrations of ethane and pentane, but not butane, are increased in the breath of currently un-medicated patients with schizophrenia, a finding that provides further evidence in support of the disease being associated with increased oxidative stress. Such a biochemical change has been suggested to be of aetiological significance in the disorder, appearing to worsen the symptoms of the disease. Being non-invasive breath alkane analysis may be suited to monitoring of this condition, although further investigation is needed to elucidate the cause of the breath changes observed.

5. ACKNOWLEDGEMENTS

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